PATENT
Docket No. GC648-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
)
Dunn-Coleman et al.)
)
Serial No. 09/991,209)
)
Filed: November 16, 2001)
)
For: Manipulation of the Phenolic Acid Content and Digestibility of Plant Cell Walls by Targeted Expression of Genes Encoding Cell Wall Degrading Enzymes)

Group Art Unit: 1645

Examiner: Unassigned

Preliminary Amendment

U.S. Patent and Trademark Office
Box Sequence, P.O. Box 2327
Arlington, VA 22202

Sir:

Prior to examination, Applicants respectfully request entry of the following amendments.

In the drawings:

Please replace Figures 45A-H with new Figures 45A-H.

In the specification:

On page 4, please replace the paragraph starting on line 4 with the following:

Figures 2 A-E illustrate the complete DNA (SEQ. ID NO:1), with highlighting to point out the signal sequence, intron and various restriction endonuclease sites, and amino acid sequence (SEQ. ID. NO:2) corresponding to the gene encoding the 38 kD ferulic acid esterase isolated from *Aspergillus niger.*

On page 4, please replace the paragraph starting on line 9 with the following:

Figure 3 illustrates the DNA sequence of the gene encoding the 38 kD esterase (SEQ. ID. NO:1).

On page 4, please replace the paragraph starting on line 13 with the following:

Figure 5 illustrates that the overlapping of PCR products made with primers FAE-I5 (SEQ ID NO:49) and FAE-I3 (SEQ ID NO:50) creates two possible uninterrupted reading frames – the top in the figure below is functional (SEQ ID NO:3) (highlighted serine is at active site), the bottom is inactivated (SEQ ID NO:4).

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Figure 9 illustrates the KDEL-COOH ER retention sequences (SEQ ID NO:6).

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Figure 10 illustrates the FAE-LINKER-FRAMESHIFT structure and sequence (SEQ ID NO:7 and 8).

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Figure 13 depicts the barley aleurain vacuolar and apoplast signal sequence (SEQ ID NO:9 and 10).

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Figure 15 illustrates the potato protease inhibitor II (PPI) motif structure and sequence (SEQ ID NO:13 and 14).

On page 6, please replace the paragraph starting on line 3 with the following:

Figure 32A is a schematic of the pTP10-1 vector. Also shown in figures 32B-32D is the 5338 bp nucleotide sequence of the vector (SEQ ID NO:15).

On page 6, please replace the paragraph starting on line 5 with the following:

Figure 33A is a schematic of the pUA4-4 vector. Also shown in figures 33B-33C is the 5345 bp nucleotide sequence of the vector (SEQ ID NO:17).

On page 6, please replace the paragraph starting on line 7 with the following:

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Figure 51 illustrates the Aleurain-NPIR delete structure. The corresponding nucleotide sequences are also shown (SEQ ID NO:45).

On page 7, please replace the paragraph starting on line 11 with the following:

Figure 52 illustrates the SEE1 (senescence enhanced) promoter sequence (SEQ ID NO:46).

On page 7, please replace the paragraph starting on line 13 with the following:

Figure 53 illustrates the SEE1 (senescence enhanced) promoter sequence plus the vacuolar aleurain signal/NPIR sequence (SEQ ID NO:47 and 48).

On page 16, please replace the paragraph starting on line 30 with the following:

The present invention provides for methods of changing the cell wall structure of transgenic plants and therefore, making them more digestible. The method comprises introducing a ferulic acid esterase coding sequence into the cells of a plant. Operably linked to the coding sequence is a promoter that can be either constitutive or inducible and signal sequences that serve to target expression of the coding sequence in the desired organelle in the desired cell of the plant. The signal sequences can be either or both N terminal or C terminal sequences.

On page 21, please replace the paragraph starting on line 27 with the following:

In addition to targeting expression to specific organelles, it may be desirable to retain the expressed FAE in the Golgi or endoplasmic reticulum. The well known ER retention signal, KDEL (SEQ ID NO:97), can be added to the 3' end of the coding polynucleotide.

On page 26, please replace the paragraph starting on line 14 with the following:

A genomic clone for FAE1 (see Figures 1-3, SEQ ID NO:1 and 2) was used as the starting point for the preparation of an intronless FAE1 encoding DNA sequence. The sequence for the genomic clone is given in Figures 2 and 3 (SEQ ID NO:1 and 2). Separate fragments for

both FAE exons were recovered by PCR from a 5.5kb EcoRI fragment of the genomic clone in pLITMUS28, and 'cDNA' created by overlapping PCR. See Figure 4.

On page 26, please replace the paragraph starting on line 20 with the following:

Two 5' primers were used. FAE-S5 which amplifies the entire reading frame (including the Aspergillus signal), and FAE-N5 which amplifies only the mature protein (i.e. has *no* signal). A number of codons are optimized (underlined in primer sequences below). The overlap product may be derived from either FAE-I5 (wild type, SEQ ID NO:49) or FAE-I3 (conserved Ser changed to Ala, SEQ ID NO:50) primers, allowing production of enzymatically inactive protein to check toxicity. As shown in Figure 5, overlapping of PCR products made with FAE-I5 and FAE-I3 creates two possible uninterrupted reading frames (SEQ ID NO:3 and 4). If the complement to FAE-I5 serves as the template when recombined then the encoded protein retains the serine moiety and the esterase is functional (highlighted serine is at active site, SEQ ID NO:95). If the FAE-I3 primer serves as the template the serine is replaced with an alanine and the esterase is inactivated (highlighted alanine in bottom amino acid sequence given in Figure 5, SEQ ID NO:96).

On page 27, please replace the paragraph starting on line 1 with the following:

Where possible, codon usage has been optimized in constructed reading frames (codon choice based on published barley preferences).

FAE-I5 (SEQ ID NO: 49)

GGCGCCGAGGGAGTGGCCGGTCACGGTCAGCGCGTAGTCC 40-mer

FAE-I3 (SEQ ID NO: 50)

CCGGCCACGCCCTCGGCGCCTCCCTGGCGGCACTC 35-mer

FAE-N5 (SEQ ID NO: 51)

CTAAAGCTTACCATGGCGGCCGCCTCCACGCAGGGCATCTCCGA 44-mer

FAE-S5 (SEQ ID NO: 83)

CTAAAGCTTAACATGAAGCAGTTCTCCGCCAA 32-mer

FAE-3 (SEQ ID NO: 52)

TCTAAGCTTGCGGCCGCGACCGGCCAGGTGCATGCGCCGCTCGTCATCCC 50-MER

On page 27, please replace the paragraph starting on line 28 with the following:

The *nos* terminator from **pMA406** (Ainley & Key (1990) PMB 14:949-60) was amplified by PCR using primers TER5 and TER3 to generate a fragment with the following sequence (SEQ ID NO:53):

(Pst1) (Not 1)
(AGACTGCAGACCATGGCGGCCGCG**K**AACCACTGAAGGATGAGCTGTAAAGAAGCAGATC
GTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATT
ATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTA
TTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAA
CAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT
CGATA AGCTT CTA GATCT (where **K**=G or T)
(HindIII) (XbaI)

On page 29, please replace the paragraph starting on line 3 with the following:

Vector sequences were confirmed by sequencing. Two artifacts were found. Firstly, the redundant codon in TER5 was found to be AAA in one clone, which was subsequently used as the source of all KDEL fusions (ie peptide sequence is KPLKDEL (SEQ ID NO:85), rather than EPLKDEL (SEQ ID NO:86) as designed). See Figure 9. Secondly, an additional base is found at the site of the redundant codon in one clone, creating a frameshifted terminal peptide (ETTEG, Figure 10 SEQ ID NO:87) which was used as a control in some constructs.

On page 30, please replace the paragraph starting on line 18 with the following:

PCR primers

TER-5 (SEQ ID NO:54)

AGACTGCAGACCATGGCGGCCGCG**KAA**CCACTGAAGGATGAGCTGTAAAGAAGCAGATC
GTTCAAACATTTG 72-MER (The KDEL stop codon is underlined.)

TER-NOT (SEQ ID NO:55)

AAGACTGCAGACCATGGCGG 20-MER

TER-3 (SEQ ID NO:56)

AGATCTAGAAGCTTATCGATCTAGTAACATAGATGACACC

ALECUT (SEQ ID NO:57)

CTAGGCGGCCGCGCGGGAGGAGGCGACGGCGAC

GLYB (SEQ ID NO:58)

GAGGGTGTATTCGGTATCGAGTTGCAGGTTCGTATC

GLY3 (SEQ ID NO:59)

CTCGATACCCATTACACCCTCACGCCTTTCGA

On page 30, please replace the paragraph starting on line 38 with the following:

i. Rice actin promoter and 1st intron

Initial vectors (Figures 11 and 12) were constructed from pCOR105 which was subsequently found to contain a 5bp deletion relative to the published sequence which destroys the AccI site (GTAGGTAGAC, SEQ ID NO:60, deleted bases underlined) and may affect splicing at the adjacent 3' site. The original rice actin sequence in this region (GTAGGTAG, SEQ ID NO:84) was therefore restored using oligonucleotide **NCO-**ACT (CTCACCATGGTAAGCTTCTACC TACAAAAAAGCTCCGCA, SEQ ID NO:61) by replacing the BglII/HindIII fragment with a PCR product, to produce vector **pPQ10.1**.

On page 32, please replace the paragraph starting on line 13 with the following:

PCR Primers

SEE-VAC (SEQ ID NO: 62)

AACCATGGCGGCCGCGCGCTCGGTGACGGGCCGGAT

SEE-NCO (SEQ ID NO: 63)

TTCGGTACCATGGCCAGGTATAATTATGG

SEE-ATG (SEQ ID NO: 64)

CTGCGCCGGCGAGATGGMCGTGCACAAGGAG

On page 32, please replace the paragraph starting on line 30 with the following:

This is from the original clone and has the peptide sequence: MKQFSAKHVLAVVVTAGHALAASTQGI (SEQ ID NO:88).

On page 33, please replace the paragraph starting on line 1 with the following:

Peptide sequence is MAAASTQGI (SEQ ID NO:89) (underlined motif is common to all constructs). Truncation of the signal sequence in (a) above was carried out by PCR with mutagenic primer FAE-N5.

On page 33, please replace the paragraph starting on line 6 with the following:

The barley aleurain vacuolar signal sequence (See Figure 13; Swissprot database accession number P05167, SEQ ID NO:10) was derived entirely from overlapping primers

(ALE-5, ALE-3, ALE-CUT ALE-CAP-5 and ALE CAP-3). Following primer annealing at 37°C and extension with T4 DNA polymerase in the presence of dNTPs according to manufacturers instructions, PCR with flanking primers ALE-5 and ALE-3 was carried out. The product was 'polished' with T4 DNA polymerase, purified, digested with NotI and cloned into EcoRV/NotI digested pCOR105-nos terminator vector (see above). ALE-3 contains redundancies so that clones encoding NPIR or NPGR motifs may be recovered. Two versions of the signal, with and without the vacuole targeting motif, were produced, to give putative vacuolar NPIR and apoplast (NPGR) signal sequences.

On page 33, please replace the paragraph starting on line 18 with the following:

PCR Primers

ALE-5 (SEQ ID NO: 65)

GGAATTCGTAGACAAGCTTACMATGGCCCACGCCCGCGTCCT 41-MER

ALE-3 (SEQ ID NO: 66)

TATCCATGGCGGCCGCGCGGTCGGTGACGGGCCGGMYCGGGTTGGAGTCGGCGAA

55-MER

ALE-CUT (SEQ ID NO: 67)

CTAGGCGGCCGCGCGGGAGGAGGCGACGGCGAC 33-mer

ALECAP-5 (SEQ ID NO: 68)

GCGACGGCGACGGCGGCCGTGGCCAGCACGGCGAGCGCCAGGAGGAGGACGCGG

54-MER

ALECAP-3 (SEQ ID NO: 69)

TCGCCGTCGCCTCCTCCTCCTCCTTCGCCGACT 33-MER

On page 34, please replace the paragraph starting on line 5 with the following:

A Golgi targeting vector, **pJQ3.2**, was made by inserting a reading frame encoding the relevant rat sialyl transferase (RST) motif (See Figure 14, SEQ ID NO:11 and 12. RST motif shown to function in plants by Boevink P, Oparka K, Cruz SS, Martin B, Betteridge A, Hawes C, (1998) PLANT JOURNAL 15 441-447 Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network) into vector pPQ10.1, and replacing the EcoRI/NotI promoter/signal fragment of **pJO6.3** with the fragment from this vector. Briefly, the RST motif was constructed by annealing oligonucleotides RST-F1A, RST-F1B, RST-F2A and RST-F2B, and amplifying the product with RST-5AD and RST-3A. This product was cloned and sequenced. Clones were

found to have a deletion which was corrected by PCR with RST-RPT, followed by overlap-PCR and cloning of products.

On page 34, please replace the paragraph starting on line 17 with the following:

PCR primers

RST-5AD (SEQ ID NO: 70)

ACTAAGCTTAAGGAGATATAACAATGATCCACACCAACCTCAA

RST-F1A **(SEQ ID NO: 71)**

TTCCATGATCCACACCAACCTCAAAAAGAAGTTCTCCCTCTTCAT

RST-F1B **(SEQ ID NO: 72)**

AGAGTGATCACGGCGAAGAGGAGGAAGACGAGGATGAAGAGGGAGAACTTCTTTT

RST-F2A **(SEQ ID NO: 73)**

TATAGATCTGCGTGTGGAAGAAGGGCTCCGACTACGAGGCCCTCACCCTCCAAGCCAAGG
A

RST-F2B **(SEQ ID NO: 74)**

CATTTGGAACTCCTTGGCTTGGAGGGTG

RST-3A **(SEQ ID NO: 75)**

AACCATGGCGGCCGCCATTTGGAACTCCTTGGCT

RST-RPT **(SEQ ID NO: 76)**

TATAGATCTGCGTGTGGAAGAAGGGCTCCGACTACGAGGCCCTCACCCTCCAAGCCAAGG
A

On page 35, please replace the paragraph starting on line 14 with the following:

PCR primers

PPI-AP1 **(SEQ ID NO: 77)**

GGAATTCGTAGACAAGCTTACMATGGMCGTGCACAAGGAGGT

PPI-AP2 **(SEQ ID NO: 78)**

GATCAGGAGGTAGGCWACGAAGTTWACCTCCTTGTGC

PPI-AP3 **(SEQ ID NO: 79)**

CCTACCTCCTGATCGTSCTCGGCCTCCTCTTGCTCGT

PPI-AP4 **(SEQ ID NO: 80)**

CCTTGGCGTCCACGTGCTCCATGGCGGAWACGAGCAAGAGGAG

PPI-AP5 **(SEQ ID NO: 81)**

GTGGACGCCAAGGCCTGCACCCKCGAGTGCGGCAACCTC

PPI-AP6 **(SEQ ID NO: 82)**
GGAATTCGCGGCCGCCGGGCAGATGCCGAAGCCGAGGTTGCCGCACT

On page 35, please replace the paragraph starting on line 31 with the following:

This was derived directly from the genomic clone (see Example 1) as a Nco1-Sph1 fragment (Sph end filled with T4 polymerase) which replaces the Nco1-Not1 region of a standard actin -FAE vector (Not1 end filled with T4 DNA polymerase).
Expression vector linker alone [CTW-PVAAA, SEQ ID NO:93] (plant optimised C-terminus for vacuole, golgi and apoplast vectors).

On page 36, please replace the paragraph starting on line 5 with the following:

CTW is the peptide sequence of the Aspergillus FAE COOH end and is here provided by oligo FAE3. In this primer the reading frame is extended to provide the additional amino acids PVAAA (SEQ ID NO:91) which are partially encoded by the Not1 site used for cloning downstream signals see c) and d) below. Some COOH amino acids /motifs may affect compartment targeting, the PVAAA (SEQ ID NO:91) sequences are expected to be neutral in this respect while the native Aspergillus end may not be.

On page 36, please replace the paragraph starting on line 12 with the following:

(c) Linker plus KPLKDEL (SEQ ID NO:90) [first K is primer artifact, intended to be E] {ER retention vectors)

On page 36, please replace the paragraph starting on line 27 with the following:

The linker used in the above C-terminal targeting sequences was PVAAA (SEQ ID NO:91).

On page 37, please replace the paragraph starting on line 22 with the following:
PCR primer
ALE-G (SEQ ID NO:92)
TATCCATGGCGGCCGCGCGGTCGGTGACGGGCCGGCCCGGGTTGGAGTCGGCGAA

In the claims:

6. The plant of claim 3, wherein the polynucleotide further comprises a polynucleotide that encodes CTWPVAAA (SEQ ID NO:93) at the 3' end.

26. The plant of claim 25, wherein the polynucleotide sequence is a KDEL sequence (SEQ ID NO:97).

28. The plant of claim 25, wherein the polynucleotide sequence is an extension of the ferulic acid esterase reading frame to provide a linker to KDEL (SEQ ID NO:97).

39. The method of claim 36, wherein the polynucleotide comprises CTWPVAAA (SEQ ID NO:93) at the 3' end.

66. The method of claim 65, wherein the polynucleotide sequence is a KDEL sequence (SEQ ID NO:97).

68. The method of claim 65, wherein the polynucleotide sequence is an extension of the ferulic acid esterase reading frame to provide a linker to KDEL (SEQ ID NO:97).

REMARKS

Entry of the above amendment prior to examination is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached pages are captioned **"Version with Markings to Show Changes Made."**

I. Amendments

The specification has been amended to correct an inadvertent omission to the figures and to place the sequence listing in the specification. Support for the amendment can be found in originally filed figures and the specification as filed.

The specification has been amended in accordance with 37 C.F.R. §1.821 through 1.825 to add the Sequence Listing.

The specification and claims have been amended in accordance with 37 C.F.R. §1.821(d) to add SEQ ID NO:s.

The specification has further been amended to correct obvious typographical errors.

The figures have been amended to correct the inadvertent omission of the DNA sequence corresponding to residues 701 – 1191 of plasmid pPQ10.1. The missing sequence is shown in the sequence of the progeny plasmid, pGT6, in Figure 41. Support for this amendment can be found at, for example, see page 31, lines 9-15, for a description of the relation between the plasmids. Figures 41B and 41C show the DNA sequence for residues 701 – 1191 of pGT6, which are identical to its parent plasmid, pPQ10.1. No new matter is introduced by this amendment.

Accordingly, in view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

The Commissioner is hereby authorized to charge the fees necessitated by the filing of these documents, or to charge any additional fees under 37 C.F.R. 1.16 and 1.17, or to credit any overpayment, to Deposit Account No. 07-1048.

If in the opinion of the Examiner a telephone conference would expedite prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 846-7615.

Respectfully submitted,

Date: June 27, 2002

Victoria L. Boyd
Registration No. 43,510

Genencor International, Inc.
925 Page Mill Road
Palo Alto, CA 94304
Phone: (650) 846-7615
Facsimile: (650) 845-6504

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The present invention provides for methods of changing the cell wall structure of transgenic plants and therefore, making them more digestible. The method comprises introducing a ferulic acid esterase coding sequence into the cells of a plant. Operably linked to the coding [seqeunce]sequence is a promoter that can be either constitutive or inducible and signal sequences that serve to target expression of the coding sequence in the desired organelle in the desired cell of the plant. The signal sequences can be either or both N terminal or C terminal sequences.

On page 21, please replace the paragraph starting on line 27 with the following:

In addition to targeting expression to specific organelles, it may be [desireable]desirable to retain the expressed FAE in the Golgi or endoplasmic reticulum. The well known ER retention signal, KDEL (SEQ ID NO:97), can be added to the 3' end of the coding polynucleotide.

On page 26, please replace the paragraph starting on line 14 with the following:

A genomic clone for FAE1 (see Figures 1-3, SEQ ID NO:1 and 2) was used as the starting point for the preparation of an intronless FAE1 encoding DNA sequence. The sequence for the genomic clone is given in Figures 2 and 3 (SEQ ID NO:1 and 2). Separate fragments for both FAE exons were recovered by PCR from a 5.5kb EcoRI fragment of the genomic clone in pLITMUS28, and 'cDNA' created by overlapping PCR. See Figure 4.

On page 26, please replace the paragraph starting on line 20 with the following:

Two 5' primers were used. FAE-S5 which amplifies the entire reading frame (including the Aspergillus *s*ignal), and FAE-N5 which amplifies only the mature protein (i.e. has *n*o signal). A number of codons are [optimised]optimized (underlined in primer sequences below). The overlap product may be derived from either FAE-I5 (wild type, SEQ ID NO:49) or FAE-I3 (conserved Ser changed to Ala, SEQ ID NO:50) primers, allowing production of enzymatically inactive protein to check toxicity. As shown in Figure 5, overlapping of PCR products made with FAE-I5 and FAE-I3 creates two possible uninterrupted reading frames (SEQ ID NO:3 and 4). If the complement to FAE-I5 serves as the template when recombined then the encoded protein retains the serine moiety and the esterase is functional (highlighted serine is at active site, SEQ ID NO:95). If the FAE-I3 primer serves as the template the serine is replaced with an alanine and the esterase is inactivated (highlighted alanine in bottom amino acid sequence given in Figure 5, SEQ ID NO:96).

On page 27, please replace the paragraph starting on line 1 with the following:

Where possible, codon usage has been [optimised]optimized in constructed reading frames (codon choice based on published barley preferences).

FAE-I5 (SEQ ID NO: 49)

GGCGCCGAGGGAGTGGCCGGTCACGGTCAGCGCGTAGTCC 40-mer

FAE-I3 (SEQ ID NO: 50)

CCGGCCACGCCCTCGGCGCCTCCCTGGCGGCACTC 35-mer

FAE-N5 (SEQ ID NO: 51)

CTAAAGCTTACCATGGCGGCCGCCTCCACGCAGGGCATCTCCGA 44-mer

FAE-S5 (SEQ ID NO: 83)

CTAAAGCTTAACATGAAGCAGTTCTCCGCCAA 32-mer

FAE-3 (SEQ ID NO: 52)

TCTAAGCTTGCGGCCGCGACCGGCCAGGTGCATGCGCCGCTCGTCATCCC 50-MER

On page 27, please replace the paragraph starting on line 28 with the following:

The *nos* terminator from **pMA406** (Ainley & Key (1990) PMB 14:949-60) was amplified by PCR using primers TER5 and TER3 to generate a fragment with the following sequence (SEQ ID NO:53):

(Pst1) (Not 1)

(AGACTGCAGACCATGGCGGCCGCG**K**AACCACTGAAGGATGAGCTGTAAAGAAGCAGATC
GTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATT
ATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTA
TTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAA
CAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGAT
CGATA AGCTT CTA GATCT (where **K**=G or T)

(HindIII) (XbaI)

On page 29, please replace the paragraph starting on line 3 with the following:

Vector sequences were confirmed by sequencing. Two artifacts were found. Firstly, the redundant codon in TER5 was found to be AAA in one clone, which was subsequently used as the source of all KDEL fusions (ie peptide sequence is KPLKDEL (SEQ ID NO:85), rather than EPLKDEL (SEQ ID NO:86) as designed). See Figure 9. Secondly, an additional base is found at the site of the redundant codon in one clone, creating a frameshifted terminal peptide (ETTEG, Figure 10 SEQ ID NO:87) which was used as a control in some constructs.

On page 30, please replace the paragraph starting on line 18 with the following:

PCR primers

TER-5 (SEQ ID NO:54)

AGACTGCAGACCATGGCGGCCGCG**KAA**CCACTGAAGGATGAGCTGTAAAGAAGCAGATC GTTCAAACATTTG 72-MER (The KDEL stop codon is underlined.)

TER-NOT (SEQ ID NO:55)

AAGACTGCAGACCATGGCGG 20-MER

TER-3 (SEQ ID NO:56)

AGATCTAGAAGCTTATCGATCTAGTAACATAGATGACACC

ALECUT (SEQ ID NO:57)

CTAGGCGGCCGCGCGGGAGGAGGCGACGGCGAC

GLYB (SEQ ID NO:58)

GAGGGTGTATTCGGTATCGAGTTGCAGGTTCGTATC

GLY3 (SEQ ID NO:59)

CTCGATACCCATTACACCCTCACGCCTTTCGA

On page 30, please replace the paragraph starting on line 38 with the following:

i. Rice actin promoter and 1st intron

Initial vectors (Figures 11 and 12) were constructed from pCOR105 which was subsequently found to contain a 5bp deletion relative to the published sequence which destroys the AccI site (GTAGGTAGAC, SEQ ID NO:60, deleted bases underlined) and may affect splicing at the adjacent 3' site. The original rice actin sequence in this region (GTAGGTAG, SEQ ID NO:84) was therefore restored using oligonucleotide **NCO-**ACT (CTCACCATGGTAAGCTTCTACC TACAAAAAAGCTCCGCA, SEQ ID NO:61) by replacing the BglII/HindIII fragment with a PCR product, to produce vector **pPQ10.1**.

On page 32, please replace the paragraph starting on line 13 with the following:

PCR Primers

SEE-VAC (SEQ ID NO: 62)

AACCATGGCGGCCGCGCGCTCGGTGACGGGCCGGAT

SEE-NCO (SEQ ID NO: 63)

TTCGGTACCATGGCCAGGTATAATTATGG

SEE-ATG (SEQ ID NO: 64)

CTGCGCCGGCGAGATGGMCGTGCACAAGGAG

On page 32, please replace the paragraph starting on line 30 with the following:

This is from the original clone and has the peptide sequence: MKQFSAKHVLAVVVTAGHALAASTQGI (SEQ ID NO:88).

On page 33, please replace the paragraph starting on line 1 with the following:

Peptide sequence is MAAASTQGI (SEQ ID NO:89) (underlined motif is common to all constructs). Truncation of the signal sequence in (a) above was carried out by PCR with mutagenic primer FAE-N5.

On page 33, please replace the paragraph starting on line 6 with the following:

The barley aleurain vacuolar signal sequence (See Figure 13; Swissprot database accession number P05167, SEQ ID NO:10) was derived entirely from overlapping primers (ALE-5, ALE-3, ALE-CUT ALE-CAP-5 and ALE CAP-3). Following primer annealing at 37°C and extension with T4 DNA polymerase in the presence of dNTPs according to manufacturers instructions, PCR with flanking primers ALE-5 and ALE-3 was carried out. The product was 'polished' with T4 DNA polymerase, purified, digested with NotI and cloned into EcoRV/NotI digested pCOR105-nos terminator vector (see above). ALE-3 contains redundancies so that clones encoding NPIR or NPGR motifs may be recovered. Two versions of the signal, with and without the vacuole targeting motif, were produced, to give putative vacuolar NPIR and apoplast (NPGR) signal sequences.

On page 33, please replace the paragraph starting on line 18 with the following:

PCR Primers

ALE-5 (SEQ ID NO: 65)

GGAATTCGTAGACAAGCTTACMATGGCCCACGCCCGCGTCCT 41-MER

ALE-3 (SEQ ID NO: 66)

TATCCATGGCGGCCGCGCGGTCGGTGACGGGCCGGMYCGGGTTGGAGTCGGCGAA 55-MER

ALE-CUT (SEQ ID NO: 67)

CTAGGCGGCCGCGCGGGAGGAGGCGACGGCGAC 33-mer

ALECAP-5 (SEQ ID NO: 68)

GCGACGGCGACGGCGGCCGTGGCCAGCACGGCGAGCGCCAGGAGGAGGACGCGG 54-MER

ALECAP-3 (SEQ ID NO: 69)

TCGCCGTCGCCTCCTCCTCCTCCTTCGCCGACT 33-MER

On page 34, please replace the paragraph starting on line 5 with the following:

A Golgi targeting vector, **pJQ3.2**, was made by inserting a reading frame encoding the relevant rat sialyl transferase (RST) motif (See Figure 14, SEQ ID NO:11 and 12. RST motif shown to function in plants by Boevink P, Oparka K, Cruz SS, Martin B, Betteridge A, Hawes C, (1998) PLANT JOURNAL 15 441-447 Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network) into vector pPQ10.1, and replacing the EcoRI/NotI promoter/signal fragment of **pJO6.3** with the fragment from this vector. Briefly, the RST motif was constructed by annealing oligonucleotides RST-F1A, RST-F1B, RST-F2A and RST-F2B, and amplifying the product with RST-5AD and RST-3A. This product was cloned and sequenced. Clones were found to have a deletion which was corrected by PCR with RST-RPT, followed by overlap-PCR and cloning of products.

On page 34, please replace the paragraph starting on line 17 with the following:

PCR primers

RST-5AD (SEQ ID NO: 70)

ACTAAGCTTAAGGAGATATAACAATGATCCACACCAACCTCAA

RST-F1A **(SEQ ID NO: 71)**

TTCCATGATCCACACCAACCTCAAAAAGAAGTTCTCCCTCTTCAT

RST-F1B **(SEQ ID NO: 72)**

AGAGTGATCACGGCGAAGAGGAGGAAGACGAGGATGAAGAGGGAGAACTTCTTTT

RST-F2A **(SEQ ID NO: 73)**

TATAGATCTGCGTGTGGAAGAAGGGCTCCGACTACGAGGCCCTCACCCTCCAAGCCAAGG
A

RST-F2B **(SEQ ID NO: 74)**

CATTTGGAACTCCTTGGCTTGGAGGGTG

RST-3A **(SEQ ID NO: 75)**

AACCATGGCGGCCGCCATTTGGAACTCCTTGGCT

RST-RPT **(SEQ ID NO: 76)**

TATAGATCTGCGTGTGGAAGAAGGGCTCCGACTACGAGGCCCTCACCCTCCAAGCCAAGG
A

On page 35, please replace the paragraph starting on line 14 with the following:

PCR primers

PPI-AP1 **(SEQ ID NO: 77)**
GGAATTCGTAGACAAGCTTACMATGGMCGTGCACAAGGAGGT
PPI-AP2 **(SEQ ID NO: 78)**
GATCAGGAGGTAGGCWACGAAGTTWACCTCCTTGTGC
PPI-AP3 **(SEQ ID NO: 79)**
CCTACCTCCTGATCGTSCTCGGCCTCCTCTTGCTCGT
PPI-AP4 **(SEQ ID NO: 80)**
CCTTGGCGTCCACGTGCTCCATGGCGGAWACGAGCAAGAGGAG
PPI-AP5 **(SEQ ID NO: 81)**
GTGGACGCCAAGGCCTGCACCCKCGAGTGCGGCAACCTC
PPI-AP6 **(SEQ ID NO: 82)**
GGAATTCGCGGCCGCCGGGCAGATGCCGAAGCCGAGGTTGCCGCACT

On page 35, please replace the paragraph starting on line 31 with the following:

This was derived directly from the genomic clone (see Example 1) as a Nco1-Sph1 fragment (Sph end filled with T4 polymerase) which replaces the Nco1-Not1 region of a standard actin -FAE vector (Not1 end filled with T4 DNA polymerase).
Expression vector linker alone [CTW-PVAAA, SEQ ID NO:93] (plant optimised C-terminus for vacuole, golgi and apoplast vectors).

On page 36, please replace the paragraph starting on line 5 with the following:

CTW is the peptide sequence of the Aspergillus FAE COOH end and is here provided by oligo FAE3. In this primer the reading frame is extended to provide the additional amino acids PVAAA (SEQ ID NO:91) which are partially encoded by the Not1 site used for cloning downstream signals see c) and d) below. Some COOH amino acids /motifs may affect compartment targeting, the PVAAA (SEQ ID NO:91) sequences are expected to be neutral in this respect while the native Aspergillus end may not be.

On page 36, please replace the paragraph starting on line 12 with the following:

(c) Linker plus KPLKDEL (SEQ ID NO:90) [first K is primer artifact, intended to be E] {ER retention vectors)

On page 36, please replace the paragraph starting on line 27 with the following:

09991209 .071202

#5

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Date: June 27, 2002 By: Carol A. See
Carol A. See

PATENT
Docket No. GC648-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
)
Dunn-Coleman et al.)
)
Serial No. 09/991,209)
)
Filed: November 16, 2001)
)
For: Manipulation of the Phenolic Acid Content)
and Digestibility of Plant Cell Walls by)
Targeted Expression of Genes Encoding Cell)
Wall Degrading Enzymes)

Group Art Unit: 1645

Examiner: Unassigned

SUBMISSION OF FORMAL DRAWINGS

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Attn: Official Draftsperson

Sir:

In response to the Notice to File Missing Parts dated January 17, 2002, please substitute the enclosed one hundred and fifty-four (154) sheets of formal drawings for the informal drawings previously filed with respect to the above-identified patent application. Should the enclosed drawings require changes, it is respectfully requested that the Patent and Trademark Office notify the undersigned attorney of same.

Respectfully submitted,

Date: June 27, 2002

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GC648-2 t-dwgs

The linker used in the above C-terminal targeting sequences was PVAAA (SEQ ID NO:91).

On page 37, please replace the paragraph starting on line 22 with the following:

PCR primer

ALE-G (SEQ ID NO:92)

TATCCATGGCGGCCGCGCGGTCGGTGACGGGCCGGCCCGGGTTGGAGTCGGCGAA

In the claims:

6. The plant of claim 3, wherein the polynucleotide further comprises a polynucleotide that encodes CTWPVAAA (SEQ ID NO:93) at the 3' end.

26. The plant of claim 25, wherein the polynucleotide sequence is a KDEL sequence (SEQ ID NO:97).

28. The plant of claim 25, wherein the polynucleotide sequence is an extension of the ferulic acid esterase reading frame to provide a linker to KDEL (SEQ ID NO:97).

39. The method of claim 36, wherein the polynucleotide comprises CTWPVAAA (SEQ ID NO:93) at the 3' end.

66. The method of claim 65, wherein the polynucleotide sequence is a KDEL sequence (SEQ ID NO:97).

68. The method of claim 65, wherein the polynucleotide sequence is an extension of the ferulic acid esterase reading frame to provide a linker to KDEL (SEQ ID NO:97).